Structure – Function Studies of Annexin A2 & A5

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Introduction:

Within the cell, proteins exist to maintain the intracellular level of calcium ions (Ca^{2+}), allowing calcium to act as a secondary messenger. Annexins are a group of these proteins located in muscle cells which bind calcium in order to bind to phospholipids in the plasma membrane. Although there are multiple forms of this protein found in humans, all contain a similar structural basis consisting of an amino-terminal head domain, a carboxyl terminal domain, and conserved structural repeats of around 70 residues. Both the calcium binding and membrane binding sites are located at the carboxyl domain. Annexins bind to negatively charged phospholipids at the cellular membranes, which differentiates them from other calcium binding proteins. Exocytosis/endocytosis, ion transport, and transport of vesicles are also processes orchestrated by these proteins.[1]

Specifically, annexin A2 (AnxA2) and A5 (AnxA5) are the proteins we studied. Figure 1 shows the 3-D structure of AnxA2 with calcium bound. The question we tried to understand is the mechanism for how AnxA2 and AnxA5 function at the membrane in terms of the redistribution of lipids and the effect on membrane permeability. In order to study the annexins, we grew E. coli cells that expressed our desired proteins. We then went through multiple week-long purification processes to obtain pure protein to be used for further studies. In the case of AnxA5, we were able to successfully purify the protein using a known protocol. For AnxA2, a purification protocol was not known; therefore one was created based on the knowledge from the AnxA5 protocol. Two purifications were completed, but neither was successful. Since AnxA2 is a membrane binding protein, it was hypothesized that the protein bound to dialysis tubing during the dialyzing step to filter out unwanted proteins and other molecules. Currently, we are trying to modify the protocol so that a successful purification can take place.

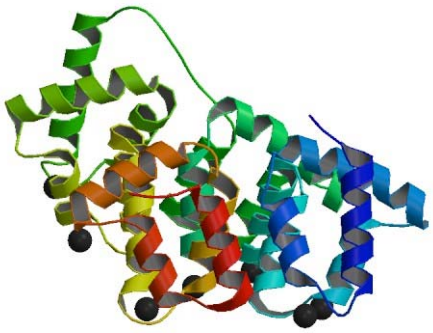

Figure 1. Structure of Annexin A2 bound to calcium.[2] Calcium is shown as the black spheres attached to the α-helicies.

In order to get an understanding of the mechanism of the annexin A5, structural and stability experiments were done. These experiments include differential scanning calorimetry (DSC), isothermal titration calorimetry (ITC), and fluorescence lifetime spectroscopy (FLT). DSC was used to determine the thermal stability and heat capacity of proteins. ITC was used to determine the change in enthalpy and binding affinity for the

interaction of molecules. FLT used fluorescence as a way to study biochemical interactions of the protein.

Figure 2 shows data obtained using FLT for annexin A5 to give information on the effects of membrane permeability. As shown, the efflux of carboxyfluorescein (CF), a dye which was monitored by the fluorescent signal given off, decreased with AnxA5 present. Therefore, annexin A5 has a direct impact on reducing the membrane permeability. The presence of cholesterol also decreased the efflux of (CF), meaning the presence of cholesterol and annexin prevents movement across the membrane.[3]

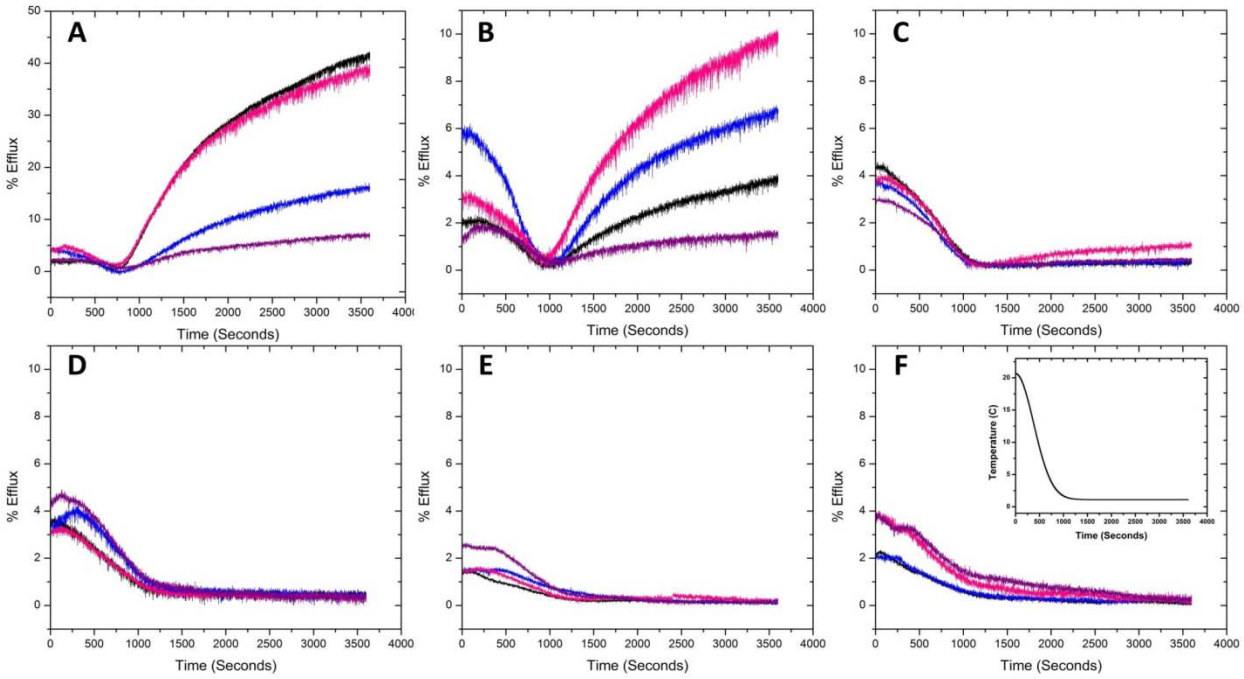

Figure 2. The efflux of carboxyfluorescein (CF) from Large Unilamellar Vesicles composed of POPC:POPS (60:40) and varying cholesterol. All samples contained 333 µM lipid and all plots are an average of three trials. Black: lipid alone; blue: lipid and Ca2+; pink: lipid and Annexin a5; purple: lipid, Annexin a5, and Ca2+. (A) POPC:POPS (60:40), no cholesterol in the vesicles; (B) POPC:POPS (60:40), with 5% cholesterol; (C) POPC:POPS (60:40), with 10% cholesterol; (D) POPC:POPS (60:40), with 20% cholesterol; (E) POPC:POPS (60:40), with 30% cholesterol; (F) POPC:POPS (60:40), with 40% cholesterol. The inset in (F) shows the decrease in temperature for all scans over 60 minutes with the water bath set to decrease from 20°C to -3°C.[3]

Other studies have looked at the binding mechanism of Ca^{2+} with AnxA5 at the membrane to test for cooperative binding. Cooperativity would occur if the binding of one calcium ion increased the binding of more calcium ions. It was shown that AnxA5 can be found in two states: the first, annexin can be free in solution, and the other can be membrane associated. The membrane associated state has higher affinity for calcium than does the solution state. Cooperative binding

of calcium only occurred if there was a low concentration of AnxA5 in the membrane bound state with no calcium initially present. Otherwise, the binding of calcium was found to be independent.[4]

From here, our goal is to modify the annexin A2 purification protocol so that further studies can be run on this protein, as well as A5.

Bibliography

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